AmoyDx® ROS1 Gene Fusions Detection Kit

For qualitative detection of 14 ROS1 gene fusions

Instruction for Use

Instruction Version: P1.1
Revision Date: December 2013

Store at -20±5°C
Background

ROS1 is a receptor tyrosine kinase of insulin receptor family. ROS1 gene fusions define a unique molecular subset of non–small-cell lung cancer (NSCLC). The ROS1 fusion partners include SLC34A2, CD74, SDC4, EZR etc. These fusions lead to constitutive kinase activity and activation of downstream pathways, such as JAK/STAT, PI3K/AKT, RAS/MAPK etc., leading to carcinogenesis. It has been reported that the presence of the ROS1 rearrangement is correlated with the efficacy of TKI therapy. Based on analysis of tumor messenger RNA, ROS1 gene fusions can be detected by real-time PCR method.

Intended Use

The AmoyDx® ROS1 Gene Fusions Detection Kit is an in vitro nucleic acid amplification test intended for qualitative detection of 14 ROS1 gene fusions in human NSCLC formalin-fixed paraffin-embedded (FFPE) tissue samples. This Kit is CE marked for IVD use in Europe and for research use only (RUO) in other countries.

Kit Contents

The kit contains sufficient reagents to carry out 12 tests (Table 1). The ROS1 gene fusions in Tubes ①~④/⑤~⑧ are indicated by FAM signal and reference gene in Tube ④/⑧ is indicated by HEX(VIC) signal.

Note: Distinguish Tube ⑧ from Tube ① according to the right middle hole of strip edge, as depicted following.

<table>
<thead>
<tr>
<th>Table 1 ROS1 Gene Fusions Detection Kit Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents Supplied</td>
</tr>
<tr>
<td>ROS1 PCR Reaction Mix</td>
</tr>
<tr>
<td>ROS1 RT Reaction Mix</td>
</tr>
<tr>
<td>ROS1 Reverse Transcriptase</td>
</tr>
<tr>
<td>ROS1 Enzyme Mix</td>
</tr>
<tr>
<td>ROS1 Positive Control</td>
</tr>
</tbody>
</table>

Equipment and Reagents Not Supplied With the Kit

1. Compatible PCR instruments: Stratagene Mx3000P™, ABI 7500, LightCycler®480 II.
   - For ABI instruments please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
   - For Stratagene Mx3000P™, if there’s low net fluorescence signal (dR) but high background signal (R), please reduces the signal gain setting of instrument properly.
   - We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

2. Nuclease-free tubes.

3. Dedicated pipette and filter pipette tips for handling RNA&DNA template.

4. Nuclease-free water.

5. RNA extraction reagents.
Shipping and Storage

The kit requires cold-chain-transportation. All contents of the kit should be stored immediately upon receipt at -20±5°C in the dark in a constant temperature freezer. Avoid unnecessary freezing and thawing of the contents of the kit. Do not use the reagent after five freeze-thaw cycles. Once opened, this reagent is stable at -20±5°C until the expiry.

Stability

The shelf-life of the kit is eight months when stored under the recommended conditions and in the original packaging. Do not use the kit after the stated expiry date.

Specimen Material

Human total RNA should be extracted from NSCLC FFPE samples with 5-10 µm thickness. The FFPE samples should be prepared and stored less than two years following proper procedures. Before extraction of RNA, it is very important to make sure that there is at least 30% tumor cells in the FFPE tissue samples. RNA should be stored below -70°C for no more than one week if no used immediately. High quality RNA is essential for the kit so we recommend use of AmoyDx RNA extraction kit (AmoyDx® FFPE RNA Kit, Cat No. ADx-FF04, for paraffin embedded specimens). The OD value of RNA samples should be measured using the spectrophotometer after extraction.

We recommend:

- Total RNA concentration should be between 50 and 800 ng/µL.
- OD_{260}/OD_{280} value should be between 1.9 and 2.1.

(Optional) Store the cDNA

Please store the cDNA at -20±5°C less than one week after reverse transcription if not proceeded to next step.

Technological Principles

The kit is intended for the qualitative detection of ROS1 gene fusions in NSCLC FFPE samples. The kit is based on three major processes: (1) specimen preparation to isolate total RNA from NSCLC FFPE samples; (2) reverse transcription of target RNA to generate complementary DNA (cDNA); (3) PCR amplification of target cDNA to detect of ROS1 gene fusions with specific primers and fluorescent probes.

Reverse Transcription

The ROS1 RT Reaction Mix contains primers specific for reverse transcription of both ROS1 RNA and reference gene RNA into cDNA.

Real-time PCR

The ROS1 PCR Reaction Mix ①~④/⑤~⑧ contains primers and FAM-labeled probes specific for ROS1 gene fusions. The ROS1 Reaction Mix ④/⑧ contains primers and HEX(VIC)–labeled probe for detection of reference gene to reveal the presence of PCR inhibitors or compromised RNA integrity that may lead to false negative results. The ROS1 Enzyme Mix contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.

Experimental Procedure

1. Reverse Transcription

(1) Thaw ROS1 RT Reaction Mix at room temperature.
(2) Centrifuge **ROS1 Reverse Transcriptase** and **ROS1 RT Reaction Mix** prior to use.

(3) For each RNA sample, transfer 0.5 μL **ROS1 Reverse Transcriptase** into an **ROS1 RT Reaction Mix** tube; mix well by pipetting gently up and down.

(4) Add 6 μL sample RNA into the appropriate centrifuge tube.

(5) Incubate the tubes at 42°C for one hour.

(6) Heat the tubes at 95°C for 5 minutes, then transfer them to ice. The resulting cDNA solutions are used for PCR amplification.

2. **PCR amplification**

(1) Thaw **ROS1 Positive Control (PC)** at room temperature.

(2) Centrifuge **ROS1 Enzyme Mix** and **ROS1 Positive Control (PC)** prior to use.

(3) Transfer 1.5 μL **ROS1 Enzyme Mix** into each cDNA, 25 μL **ROS1 Positive Control (PC)**, 25 μL ddH₂O (NTC).

(4) Mix well by pipetting gently up and down and spin for 5 seconds.

(5) Add 5 μL sample cDNA Mix, 5 μL ROS1 Positive Control Mix or 5 μL ddH₂O Mix to the appropriate PCR reaction tubes. Add the mix to the side of the tube wall above the reagents in the tube.

(6) Seal the strips and spin the PCR tubes gently to collect the reagents at the bottom of tubes.

**Note:** this spin step is essential for proper mixing of the reagents.

(7) Place the PCR tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 2.

**Note:** place the PCR tubes into the real-time PCR instrument and start to run immediately. If not, please store the PCR tubes at 4°C for no more than 12 hours.

**Table 2 Suggested Plate Layout (For 12 tests)**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Signal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>①</td>
<td>FAM</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>Sample 5</td>
<td>Sample 7</td>
<td>Sample 9</td>
<td>Sample 11</td>
<td>NTC</td>
</tr>
<tr>
<td>②</td>
<td>FAM</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>Sample 5</td>
<td>Sample 7</td>
<td>Sample 9</td>
<td>Sample 11</td>
<td>NTC</td>
</tr>
<tr>
<td>③</td>
<td>FAM</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>Sample 5</td>
<td>Sample 7</td>
<td>Sample 9</td>
<td>Sample 11</td>
<td>NTC</td>
</tr>
<tr>
<td>④</td>
<td>FAM &amp; HEX/VIC</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>Sample 5</td>
<td>Sample 7</td>
<td>Sample 9</td>
<td>Sample 11</td>
<td>NTC</td>
</tr>
<tr>
<td>⑤</td>
<td>FAM</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>Sample 6</td>
<td>Sample 8</td>
<td>Sample 10</td>
<td>Sample 12</td>
<td>PC</td>
</tr>
<tr>
<td>⑥</td>
<td>FAM</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>Sample 6</td>
<td>Sample 8</td>
<td>Sample 10</td>
<td>Sample 12</td>
<td>PC</td>
</tr>
<tr>
<td>⑦</td>
<td>FAM</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>Sample 6</td>
<td>Sample 8</td>
<td>Sample 10</td>
<td>Sample 12</td>
<td>PC</td>
</tr>
<tr>
<td>⑧</td>
<td>FAM &amp; HEX/VIC</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>Sample 6</td>
<td>Sample 8</td>
<td>Sample 10</td>
<td>Sample 12</td>
<td>PC</td>
</tr>
</tbody>
</table>

(8) Carry out real-time PCR procedure using the cycling conditions described in Table 3.

**Note:**

- The reaction volume is 40 μL per well.
- Please pack the post-PCR tubes with two disposable gloves and discard properly. Do NOT open the post-PCR tubes to avoid contamination.
Table 3 PCR Cycling Parameters

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95°C</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>95°C</td>
<td>25s</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>64°C</td>
<td>20s</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>20s</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60°C</td>
<td>35s</td>
<td>☆Data collection of FAM and HEX(VIC)</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>20s</td>
<td></td>
</tr>
</tbody>
</table>

Sample Data Analysis

1. The FAM signal indicates the sample ROS1 gene fusions status in Tube ①~④/⑤~⑧ and the HEX(VIC) signal indicates the reference gene RNA status in Tube ④/⑧.
2. Ensure the calibration fluorescence is unselected. Select the sample and controls as a group, and then adjust the threshold separately for FAM and HEX/VIC amplification curves to obtain the Ct values of the samples.
3. Assess each NTC Ct value to ensure that there is no positive amplification, if the NTC has positive amplification, the data must be discarded as there may be contamination.
4. Assess each Positive Control (PC) Ct value to ensure there is a positive amplification, and the Ct value should be less than 24. Otherwise, the data must be discarded.
5. Analysis of the sample reference gene assay (HEX/VIC), there are 3 possible outcomes:
   a) If the sample reference gene Ct value ≤ 20, then continue with the analysis.
   b) If the sample reference gene Ct value > 20 which indicates the partial fragmentation or degradation of RNA, but if the FAM Ct value located in positive area, the result is believable, otherwise, we suggest re-extracting the RNA and doing this experiment again.
   c) If the sample reference signal assay fails, the data must be discarded as there may be inhibitors present, or the sample RNA has been fragmented or degraded which is not suitable for the kit. Please re-extract the RNA and do this experiment again.
6. Analysis of fusion assay results, there are 2 possible outcomes:
   a) If the sample FAM Ct value ≥ 30, the sample contains no ROS1 gene fusion.
   b) If the sample FAM Ct value < 30, the sample contains ROS1 gene fusion.
7. The sample may contain two or more fusion patterns simultaneously.

Warnings and Precautions

1. Please read the instruction carefully and become familiar with all components of the kit prior to use.
2. The product specified above does not contain any virus, reagent by-product of the same, or metabolic by-product of Hepatitis A, B, C, D or HIV.
3. Do not exchange and mix up the kit contents with different batches.
4. The kit and its contents cannot be resold or modified for re-sale without the written approval of the manufacturer.
5. Using other sources of reagents is not recommended. Strictly distinguish the reagents from positive control to avoid contamination. Otherwise, false positive may be produced.
6. Do the experiments with attention to prevent exogenous nuclease contamination to reagents. It is recommended that users have separate, dedicated pipettes and filter pipette tips to add nucleic acid template during the preparation of reagents.

7. To optimize the activity and performance, mixtures should always be protected from light to avoid photo bleaching.

8. Only trained professionals could use this kit. Please wear suitable lab coat and disposable gloves. The used kit should be disposed of properly.

Notes

1. ![EC REP](symbol) Symbol for "AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY"

2. ![IVD](symbol) Symbol for "IN VITRO DIAGNOSTIC MEDICAL DEVICE"

3. ![Umbrella](symbol) Symbol for "KEEP DRY"

4. ![Up Arrow](symbol) Symbol for "THIS WAY UP"

5. ![Glass](symbol) Symbol for "FRAGILE, HANDLE WITH CARE"

Information of European Authorised Representative

Wellkang Ltd t/a Wellkang Tech Consulting Suite B, 29 Harley Street, London W1G 9QR United Kingdom

References


Appendix I

**ROS1** gene fusions detected with the Kit

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Spliced Gene &amp; Exon</th>
<th>ROS1 Spliced Exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SLC34A2 exon4, SDC4 exon2</td>
<td>CD74 exon6</td>
</tr>
<tr>
<td></td>
<td>SLC34A2 exon14del, SDC4 exon4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SLC34A2 exon4, SDC4 exon4</td>
<td>CD74 exon6</td>
</tr>
<tr>
<td></td>
<td>SLC34A2 exon14del, EZR exon10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TPM3 exon8, LRIG3 exon16</td>
<td>GOPC exon8</td>
</tr>
<tr>
<td>4</td>
<td>GOPC exon4</td>
<td></td>
</tr>
</tbody>
</table>